

ENHANCING THE BIOLOGICAL CONTROL POTENTIAL OF
ENTOMOPATHOGENIC NEMATODES PROTECTION FROM
DESICCATION AND UV RADIATION

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ABSTRACT

Entomopathogenic nematodes (EPNs) are obligate parasites of insects. EPNs have a broad host range, are easily mass reared, and kill insects within 48 hours. EPNs are safe for vertebrates, plants, and non-target organism. On the other hand, EPNs have disadvantages that make them less effective against foliar insect pest because they are sensitive to desiccation, ultraviolet (UV) radiation and high temperatures. The goal of this research was to improve the efficacy of aboveground application EPNs by protecting them against desiccation and UV radiation. The first objective was to determine efficacy of the desiccation protectant Barricade gel in extending the viability of EPNs. The second objective was to evaluate the effect of UV chemical protectants on EPNs viability. The third objective was to demonstrate enhanced insect control with EPNs protected from UV radiation and desiccation.

Barricade® is a proprietary fire-protection product that prevents desiccation. Barricade® gel toxicity to *Steinernema feltiae* and mealworm (*Tenebrio molitor*) was determined in laboratory experiments. Subsequently, the effect of Barricade® gel on IJ infectivity was determined at different (0, 1, 1.5, 2, 3, and 4%) concentration. Barricade® gel was not toxic to mealworm larvae nor the IJ of *S. feltiae*. IJ survival was enhanced by Barricade® gel and Barricade® gel prevented desiccation of IJs allowing for greater IJ infection and mealworm larvae mortality. In an excised leaf experiment, Barricade® application enhanced IJ infection of the mealworm larvae over time compared to application in water alone.

EPNs and mealworm larvae were exposed to UV radiation protectant chemicals P-amino benzoic acid (PABA) and octyl methoxycinnamate (OMC), Congo red, titanium dioxide, and zinc oxide were not toxic to mealworm larvae and EPNs. PABA, OMC, Congo red, titanium dioxide,

and zinc oxide protected IJ from exposure to UV light for 0, 4, 8 and 12 hrs. After 48 hrs exposure, PABA afforded the greatest protection to EPN compared to other chemicals. OMC provided the second greatest protection to EPN compared to other chemicals.

The UV protectants PABA and OMC were individually combined with Barricade® fire gel and IJs were added. The IJs were exposed to full spectrum UV light in the laboratory for up to 12 hrs. The subsequent number of dead mealworm larvae was greatest in the combination treatments of OMC+Barricade and PABA+Barricade. These treatments afforded the greatest protection to IJs from UV radiation and desiccation in a filter paper experiment and a choy sum (*Brassica chinensis* var. *parachinensis*) leaf assay.

The survival of IJ can be enhanced when applied with a desiccant protectant such as Barricade and a chemical such as PABA or OMC to protect against UV radiation. The protection afforded to the IJ by these chemicals allows for greater infection and mortality of target insect pests. The formulation of IJs with desiccant and UV radiation protection will allow the greater use of EPN for the management of foliar insect pests.

Keywords: Entomopathogenic Nematodes, UV Radiation, Desiccation

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CHAPTER 1

ENTOMOPATHOGENIC NEMATODES

INTRODUCTION

Entomopathogenic nematodes (EPNs) are non-segmented roundworms that are obligate parasites of insects. Nematodes with entomopathogenic behavior have been described in 23 different nematode families (Table 1.1). Seven of these families have potential for biological control of insects: the Mermithidae, Tetrandonematidae, Allantonematidae, Sphaerulariidae Phaenopsitylenchidae, Heterorhabditidae, and Steinernematidae (Koppenhofer, 2007).

As obligate parasites, EPNs offer an alternative management tactic for many different insects (Lewis and Clarke, 2012; Shapiro-Ilan *et al.*, 2014). EPNs are advantageous biological control agents because they infect and kill a wide range of insect (Georgis *et al.*, 1991). EPNs are able to infect more than 200 insects under laboratory conditions, some of which are not soil-borne (Hazir *et al.* 2003). EPNs are not harmful to humans, other animals, or plants (Akhurst and Smith, 2002; Denno *et al.*, 2008). Whereas fungal, bacterial, and arthropod biological agents need days or weeks to kill the insect host, EPNs kill the insect within 24-48 hours from infection. EPNs are easy to reproduce and inexpensive to culture (Kaya and Gaugler, 1993). Application of EPNs does not require special personal protective equipment. EPNs occur in a variety of soil types and environments (Burnell and Stock, 2000; Georgis *et al.* 1991). Consequently, EPNs have great potential to suppress above ground insect pests via foliar application (Arthrus *et al.*, 2004; Shapiro-Ilan *et al.*, 2006).

Table 1.1 Families of nematodes in which entomopathogenic nematodes species have been identified (Koppenhofer, 2007).

Nematode families		
Anguinidae	Itonchidae	Rhabditidae
Allantonematidae	Mermithidae	Sphaerulariidae
Alloionematidae	Neotylenchidae	Steinernematidae
Diplogasteridae	Oxyuridae	Strongyloididae
Diplogasteroididae	Panagrolaimidae	Tetradonematidae
Heterorhabditidae	Phaenopstylenchidae	Thelastomatidae
Hystrignathidae	Protelloididae	Travassosinematidae
Tylenchidae		

EPNs in the Heterorhabditidae, and Steinernematidae share a similar life cycle across species. The nematode eggs hatch and proceed through four juvenile stages before reaching the adult (Poinar 1993). The third-stage juvenile (J3) enters into a dauer larvae state and is called the Infective Juvenile (IJ).

IJs survive in soil, enter, infect insect hosts (Poinar, 1990). The IJ is the only free-living stage found in EPNs. All other life stages occur within the insect host. IJ enter an insect host via the spiracles, the mouth, the anus or, in some species, through the intersegmental membranes of the cuticle (Lewis and Clarke, 2012). After infection, EPNs release symbiotic bacteria into the insect host. *Xenorhabdus* and *Photorhabdus* are the symbiotic bacteria associated with Steinernema and Heterorhabditis, respectively (Ferreira and Malan, 2014). These are gram-negative bacteria of the family Enterobacteriaceae (Boemare *et al.*, 1993). IJs carry symbiotic bacteria in their midguts (Dilman *et al.*, 2012). Bacteria kill the host insects via direct infection

through the promotion of the secondary metabolites and toxins produced by bacteria (Kaya and Gaugler, 1993). The toxins or secondary metabolites are produced by symbiotic bacteria, killing the insects by induction of immunosuppression and pervasion of the hemolymph (Park and Kim, 2000). As a result, the symbiotic bacteria replicate rapidly and cause septicemia in insects (Forst *et al.*, 1997).

The infected insect dies within 24 to 48 hours from septicemia. The EPNs feed on the bacteria and the insect guts. The EPNs reproduce one or more generations inside the insect cadaver. The number of generations depends upon on resources provided by the cadaver. As nutrients become limiting and toxic products accumulate within the insect cadaver, the J2 switches from development into J3 but into the production of the dauer stage IJ. The IJs exit the cadaver and move into the environment in search of a new host (Kaya and Gaugler 1993).

Species in the families Steinernematidae and Heterorhabditidae have been used most commonly as a biological control agent (Grewal *et al.*, 2005). Numerous examples of successful deployment of EPNs exist. Recent biological control successes include *Heterorhabditis bacteriophora* and *Steinernema rarum* infecting *Diloboderus abderus* (Coleoptera: Scarabaeidae) larvae on wheat (*Triticum aestivum*) (Eleodoro *et al.*, 2017). Shapiro-Ilan *et al.* (2013) showed that *S. riobrave* and *S. feltiae* have high virulence against plum curculio, *Conotrachelus nenuphar*, a major pest of stone and pome fruits. *Steinernema glaseri* is used for control of Japanese beetle, *Popillia japonica*, an important pest of turf in the US (Gaugler *et al.*, 1992). *Steinernema scarabaei* (100%), *H. bacteriophora* strain GPS11 (34–97%), *H. bacteriophora* strain TF (65–92%), and *H. zealandica* strain X1 (73–98%) have provided field control of *P. japonica* (Grewal *et al.*, 2005; Koppenhofer *et al.*, 2006). Black vine weevil, *Otiorhynchus sulcatus* (Curculionidae), is a pest of small fruit crops, ornamentals, and turf has been controlled by *S. carpocapsae* and *H.*

megidis in strawberry plantings. *Steinernema carpocapsae* treatment in late summer resulted in a 49.5% reduction of early instar *O. sulcatus* larvae, and late spring application resulted in 65% control of late instars (Kakouli *et al.*, 1997).

Fungus gnats, *Lycoriella* spp. (Sciaridae), are important pests in mushroom cultivation and in greenhouse plant production. *Steinernema feltiae* and *Heterorhabditis* spp. are effective for control of *L. auripilla*, *L. mali*, *L. solani* (Tomalak *et al.*, 2005; Grewal, 2007). Baur *et al.* (1998) demonstrated that *S. carpocapsae* provided 58% control of diamondback moth larvae, *Plutella xylostella* (Plutellidae). However, foliar applications of *S. carpocapsae* did not provide sufficient control of cabbage worm *Artogeia rapae* (Pieridae) under environmental conditions in Québec (Belair *et al.*, 2003).

Although EPNs have many advantages as a biological control agent, they also have drawbacks. Application of EPNs to control foliage-feeding insects has been disappointing since the mid-1950s (Kaya, 1985; Begley, 1990). Efficacy of foliar application of EPNs is affected by environmental conditions such as desiccation, temperature, and ultraviolet radiation (UV) (Shapiro-Ilan *et al.*, 2006; 2012). EPNs are adversely affected by high ambient temperatures, especially in tropical regions, and foliar applications (Kamionek *et al.*, 1974). Foliar application of EPNs is limited by UV to which they have little tolerance (Walia and Nandal, 2008). EPNs also have low tolerance to rapid desiccation (Nimkingrat *et al.*, 2013). Combined, these factors have limited the ability of EPNs to be employed as biological control agents against foliar insect pests.

A variety of commercial products are available that limit water loss or prevent damage from UV. For example, paraffin oil, alginate, carbonxy methylcellulose, guar gum, Arabic gum, and xanthan are used to retain moisture in different applications with nematodes (Beck *et al.*, 2013) (Saleh *et al.*, (2015). These products may have potential use with EPN to address and alleviate the

loss of water and desiccation EPNs encounter during above ground applications for biological control. Schroer *et al.* (2005) demonstrated that guar gum afforded some protection to EPNs compared to no desiccation protectant. Saleh *et al.* (2015) demonstrated that paraffin oil, alginate, and xanthan had some positive effect in protecting EPNs from desiccation.

A wide range of chemicals absorb or block UV radiation. These chemicals are commonly used in sunscreens for humans. UV absorbing compounds include P-amino benzoic acid (PABA) and octyl methoxycinnamate (OMC). PABA is an organic compound that is also known as 4-aminobenzoic acid and para-aminobenzoic acid. PABA is widely used in sunscreens as a UV filter. PABA was one of the first active ingredients to be used in a sunscreen in 1943 (Mitchnick *et al.*, 1998). PABA reduces UV damage through its absorption of UVB (Thune *et al.*, 1990). PABA absorbs wavelengths between 290 and 320 nm (Arumanayagam and Murugakoothan, 2011) and was found to be effective in reducing the harmful effects of UV on EPNs (Gaugler and Boush, 1979; Hussaini *et al.*, 2003). Walia *et al.* (2008) showed that a 0.05% concentration of PABA protected EPNs exposed to the sun for 60 min, after which the EPNs killed 100% of exposed *Galleria* larvae. OMC is an organic ester formed from methoxycinnamic acid and 2-ethylhexanol (Nash, 2006). It is a clear liquid that is insoluble in water. OMC is the most widely used sunscreen agent (Scalia *et al.*, 2002). Danica *et al.* (2016) showed that OMC is not toxic to EPNs. After 120 min sun exposure, 80% of EPNs remained viable.

Congo red, titanium dioxide, and zinc oxide are UV blockers. Congo red is a water-soluble organic benzidine-based anionic diazo dye. Congo red has been effective in protecting gypsy moth nuclear polyhedrosis virus from UV radiation (Shapiro, 1989) and has excellent absorbance of UVA and UVB radiation. Walia *et al.* (2008) demonstrated that after UV exposure, *Steinernema* (RB-5) treated with Congo red had 100% survival and *H. bacteriophora* had up to

96% survival compared to untreated controls. EPNs exposed to Congo red remained infective on larvae of *Galleria*. Titanium dioxide, also known as titanium (IV) oxide or titania, is a naturally occurring oxide of titanium, TiO_2 (Dransfield, 2000). Microsized TiO_2 has been used as a particulate sunscreen ingredient (average size 0.1–10.0 μm) for more than 15 years (Gasparro *et al.*, 1998). Walia *et al.* (2008) demonstrate that after 60 min. UV exposure, *H. bacteriophora*, *Steinernema* isolate Janti and *Steinernema* isolate RB-5 treated with TiO_2 survived at 100, 76 and 40.4% respectively. Zinc oxide (ZnO) is an inorganic compound that occurs in nature as the mineral zincite (Liedekerke, 2006). Pure ZnO is a white powder, but in nature it occurs as the rare mineral zincite, which usually contains manganese and other impurities that confer a yellow to red color (Kligshirn, 2007). Zinc oxide can be used in creams and lotions to protect against sunburn and other damage to the skin caused by UV (More, 2007). Zinc oxide provides the broadest spectrum UVA and UVB absorber and is completely photostable (Mitchnick *et al.*, 1999). Walia *et al.* (2008) demonstrated that after UV exposure, EPNs treated with zinc oxide received some protection. After 60 min UV exposure, *H. bacteriophora* had a 26% survival rate. *Steinernema* isolate Janti and *Steinernema* isolate RB-5 had only a 1.7 and 0.9 % survival rate respectively.

OBJECTIVES

The goal for my research was to improve the efficacy of aboveground and foliar applications of EPNs. Improvement can be accomplished by providing EPNs protection from desiccation and UV light. The first objective of my research was to determine efficacy of the desiccation protectant, Barricade[®], in extending the viability of EPNs. The second objective of my research was to evaluate the effect of UV chemical protectants on EPNs viability. The third

objective was to demonstrate enhanced insect control with EPNs protected from UV and desiccation.

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CHAPTER 2

PROTECTION OF *STEINERNEMA FELTiae* FROM DESICCATION WITH THE FIRE PROTECTANT BARRICADE

INTRODUCTION

EPNs survival depends on abiotic and biotic factors in the environment (Glazer, 1996 and, Kaya 1990). EPNs are well adapted to underground environments, but less adapted to above-ground conditions. Above ground, EPNs are subjected to sunlight and drying. Surrey and Wharton (1995) demonstrated that EPN survival was poor once water had been lost from the substrate. Sunlight can dramatically decrease *Steinernema* spp.'s pathogenicity and survival (Gaugler *et al.*, 1991). Furthermore, EPNs have low tolerance to desiccation (Nimkingrat *et al.*, 2013). Consequently, when applied above-ground, EPNs require protection against desiccation.

A variety of commercial products are available as desiccation protectants. Protectants include paraffin oil, alginate, carbonxy methylcellulose, guar gum, Arabic gum, xanthan and all have been tested on nematodes (Beck *et al.*, 2013) (Saleh *et al.* (2015). These products may have potential use with EPNs to address and alleviate the loss of water and desiccation of the nematode during above-ground applications for biological control. Some research suggests that these chemicals are mostly ineffective in protecting EPNs against desiccation. Schroer *et al.* (2005) demonstrated that Arabic gum had no protective effect on EPNs, whereas Guar gum afforded some protection to EPNs compared to no desiccation protectant. Saleh *et al.* (2015) demonstrated that paraffin oil, alginate, and xanthan had some positive effect in protecting EPNs from desiccation.

Barricade® gel is a liquid concentrate which, when mixed with water, provides fire protection (http://firegel.com/Barricade_Gel.aspx). Barricade® is a proprietary product made from

absorbent polymers (probably sodium polyacrylate). The protective coating provided by Barricade® can last for up to 24 hrs, depending on weather conditions (temperature, wind and humidity). Misting the coating with additional water spray can extend the protection times (Barricade® gel, 2006).

Barricade® gel has been effective in improving EPN control of insects (Shapiro-Ilan *et al.*, 2015). EPNs applied in a Barricade® solution were protected from desiccation and possibly from harmful UV radiation resulting in greater control of the lesser peachtree borer, *Synanthedon pictipes* (Shapiro-Ilan *et al.*, 2010). Shapiro-Ilan *et al.* (2010) showed that *Steinernema carpocapsae* applied with 2-4% Barricade® increased efficacy against *S. pictipes*. The EPNs plus Barricade reduced *S. pictipes* survival to 0-30%. The EPN without Barricade® treatment did not affect larvae survival. Shapiro-Ilan *et al.* (2010) documented that Barricade® provided moisture on the exposed tree surface and temporarily facilitated nematode deposition, survival, and host invasion. Shapiro-Ilan *et al.* (2016) demonstrated that when applied with 2% Barricade, *S. carpocapsae* lowered populations of *S. pictipes* more than when applied with just water.

Danica *et al.* (2016) showed that Barricade® is not toxic to IJs of *S. carpocapsae*. Survival of the IJs was not different from a water control. In addition, the ability of EPNs to infect and kill a host after exposure to the sun was greater (50-80%) for 1 and 2% Barricade® gel solutions than only water. Furthermore, exposure of EPNs in a 1% Barricade® gel solution to sunlight for 60 min resulted in greater host mortality than in EPNs in water only. Danica *et al.* (2016) also concluded that Barricade® allowed the EPNs to stick to the leaf more efficiently than when applied with only water.

Barricade® shows promise as a protectant that can increase the biological control potential of EPNs against above-ground pests. However, Barricade® has been evaluated on a limited number

of EPN species. The objective of this experiment was to determine the level of protection Barricade® affords *S. feltiae*.

MATERIALS AND METHODS

Steinernema feltiae was cultured in commercially obtained mealworm larvae (*Tenebrio molitor*) (Fig. 1.1). Ten mealworm larvae were placed in 100-mm-d petri dishes lined with Whatman #1 filter paper. The paper was moistened with 1 ml of a solution containing 1000 IJ of *S. feltiae* per dish. Additional water was added to ensure the paper was entirely moist. After 48 hrs in the laboratory at 25°C, dead larvae were moved to White traps (White, 1927) and held at 25°C until IJs emerged. Newly emerged IJs were collected daily, pooled, counted, and adjusted to 1,000 IJ/ml. These IJs were stored at 15°C until used in the following studies. No cultures used in this experiment were over 30-day old.

In a preliminary experiment, mealworm larvae (*Tenebrio molitor*) exposed to Barricade® gel to determine possible toxicity. Five mealworm larvae were placed in 100-mm-d petri dishes lined with Whatman #1 filter paper. The paper was moistened with 1 ml of 0, 0.5, 1 and 2% Barricade® gel solution exposed to mealworm. After 24 and 48 hrs in the laboratory at 25°C, dead larvae were counted. Each concentration was replicated in 4 plates. The experiment was repeated three times. Percent mortality was calculated and analyzed for variance. Analysis of variance (ANOVA) were conducted using JMP statistical software (SAS Inc., Cary, NC).

Toxicity: *Steinernema feltiae* IJs were exposed to Barricade® gel in the laboratory. Before each experiment, the desired Barricade® gel solutions were made, and 1 ml transferred to a 100-mm-d petri plate. A 1 ml aliquot of 1,000 IJ was added to 0, 0.5, 1 and 2% Barricade® gel solutions. The petri plates were held for 6 hrs in the dark at 25°C after which the EPN were evaluated for

mortality. Twenty IJs were pipetted into a BPI dish and EPN survival was assessed by movement response when probed with a dissecting needle (Kaya and Stock, 1997). A single plate was served as an observation. Each concentration was tested in 4 plates, with each plate serving as a replication. The experiment was repeated three times. Percent mortality was calculated and analyzed for variance. A Chi-Square test for homogeneity of variance among the three trials and ANOVA among treatments were conducted using JMP statistical software (SAS Inc., Cary, NC).

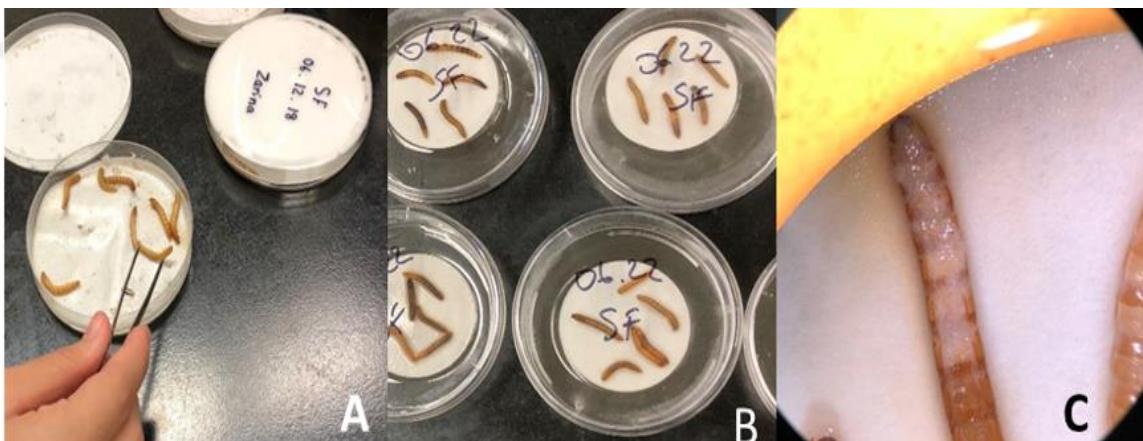


Fig. 2.1. Culturing of the entomopathogenic nematode *Steinernema feltiae*. (A) Mealworms are exposed to nematodes in petri dishes and after 48 hrs, dead mealworms are collected; (B) transferred to White trap until (C) Infective Juveniles leave the mealworm cadavers.

Effects on IJ infection: The effect of Barricade® gel on IJ infectivity was determined. Solutions of 0, 1, 1.5 and 2% Barricade® gel containing 1000 IJ/ml were made. These solutions were sprayed onto Whitman #1 filter paper disks which were then placed in 60-mm-d petri plates. The dishes were left open to dry on a laboratory bench for 1, 2, 3, 4, 5, or 6 hrs after which 3 mealworm larvae were introduced into each dish. The dishes were closed and after 48 hrs exposure, the number of dead mealworm larvae was determined. Larval death was confirmed by a movement response. Each treatment, represented by a plate containing three mealworm larvae, was replicated

four times and the entire experiment was repeated three times. A Chi-Square test for homogeneity of variance among trials and subsequent ANOVA were conducted using JMP statistical software.

In an additional experiment, IJ survival at higher Barricade® gel concentrations were evaluated. Solutions of 0, 2, 3, and 4% Barricade® containing 1000 IJ/ml were made and sprayed on Whitman #1 filter paper disks. The disks were then placed in 60-mm-d petri plates and left open for 1, 2, 3, 4, or 5 hrs on a laboratory bench after which 3 mealworms were introduced to each dish. After 48 hrs exposure to EPN, mortality of mealworms was determined. Each treatment, represented by a plate containing three mealworm larvae, was replicated four times and the entire experiment was repeated three times. A Chi-Square test for homogeneity of variance and ANOVA were conducted using JMP statistical software (SAS).

In a third experiment designed to mimic the leaf environment, a single leaf of *Brassica chinensis* var. *parachinensis* (choy sum) was placed in a 60-mm-d petri dish. The leaves were sprayed with a 0, 0.5, 1 or 2% solution of Barricade® gel containing 1000 IJ/ml. The petri plates were allowed to air dry for 1, 2, 3, 4, or 5 hrs on a laboratory bench. After each drying period, three mealworm larvae were introduced into each dish. After 48 hrs exposure to the EPN, mortality of the mealworm larvae was determined by movement response. The number of dead larvae was recorded. Each treatment, represented by a plate containing three mealworm larvae, was replicated four times and the entire experiment was repeated three times. Data was tested for homogeneity of variance among trials, and difference among treatments determined using JMP statistical software (SAS).

RESULTS

Barricade® gel was not toxic to mealworm larvae. All mealworms were alive at the 0 % Barricade® concentration at 24 hr. In all other concentrations (0.5, 1 and 2%), over 95% of mealworms were alive at 24 hr. The percentage of live mealworm decreased at 48 hr compared to 24 hr. Some mortality was recorded in the 0% Barricade® treatment (6%) compared to 6, 4, and 6 % mortality at 0.5, 1, and 2% respectively (Fig. 2.2). There was no difference detected between 24h and 48h of exposing EPNs to different concentration of Barricade treatment as indicated by statistical analysis (Fig. 2.2; $P > 0.05$)

Barricade® gel did not adversely affect EPN infection nor the EPNs' ability to infect and kill mealworm larvae. In the first experiment, IJ survival was enhanced by Barricade®. Survival of IJs differed between hours 1, 2, and 3 and hours 4, 5, and 6. IJs in the first 3 hrs had greater survival than those from the later3 hours ($P \leq 0.0005$). IJ survival was 100% for first 3 hours but after 3 hrs IJ survival decreased to 85% ($P \leq 0.0001$) (Fig. 2.3). IJ survival increased over time with increasing Barricade® concentration. The 1% Barricade® treatment had the greatest number of living IJ compared to other concentration of Barricade ($P \leq 0.0001$) (Fig. 2.4). The higher concentrations of Barricade® gave similar results. The higher Barricade® concentrations were not toxic to the IJs nor did it negatively impact IJ infectivity. Less insect mortality was recorded in the water treatment (0% Barricade®) than in other treatments (Fig. 2.5) ($P \leq 0.0001$). At 48 hrs, all treatments caused higher mortality of IJs between 75 to 90 % (Fig. 2.6). However, at 53 hrs, IJ exposed to 10 % Barricade® decreased survival to 7 % while 38 % Barricade® had still 25 % survival (Fig. 2.6) ($P \leq 0.0001$).

Barricade® gel prevented desiccation of IJs and allowed for greater mealworm larvae mortality. Insect mortality after 48 hrs was greater in the Barricade® treatments (0.5, 1 and 2 %)

than the water control treatment ($P \leq 0.0001$) (Fig. 2.7). Larval mortality was high, between 90-100% at 48 hrs in all treatments than at 0 hrs (Fig. 2.8). As the desiccation time increased, larval mortality gradually decreased to between 70 and 8% by 6 hrs. Insect mortality decreased the most, from 90% to 8% in 6 hrs, in the water only treatment (0% Barricade). The greatest mealworm mortality was recorded in the 2% Barricade[®] treatment (Fig. 2.8). A significant difference in insect mortality was evident among treatments with Barricade[®] (0.5, 1, and 2%) compared to water only treatment ($P \leq 0.0001$) (Fig. 2.8).

In the detached leaf experiment, Barricade[®] enhanced IJ infection on the mealworm larvae as the desiccation time increased. At 0 hr, all IJ treatments resulted in insect mortality between 90-100% ($P \leq 0.0001$) (Fig. 2.9). As desiccation time increased, insect host mortality gradually declined to 5% in the water treatment (Fig. 2.10). After 5 hrs of desiccation, the 2% Barricade[®] treatment only resulted in 68% larval mortality (Fig. 2.10).

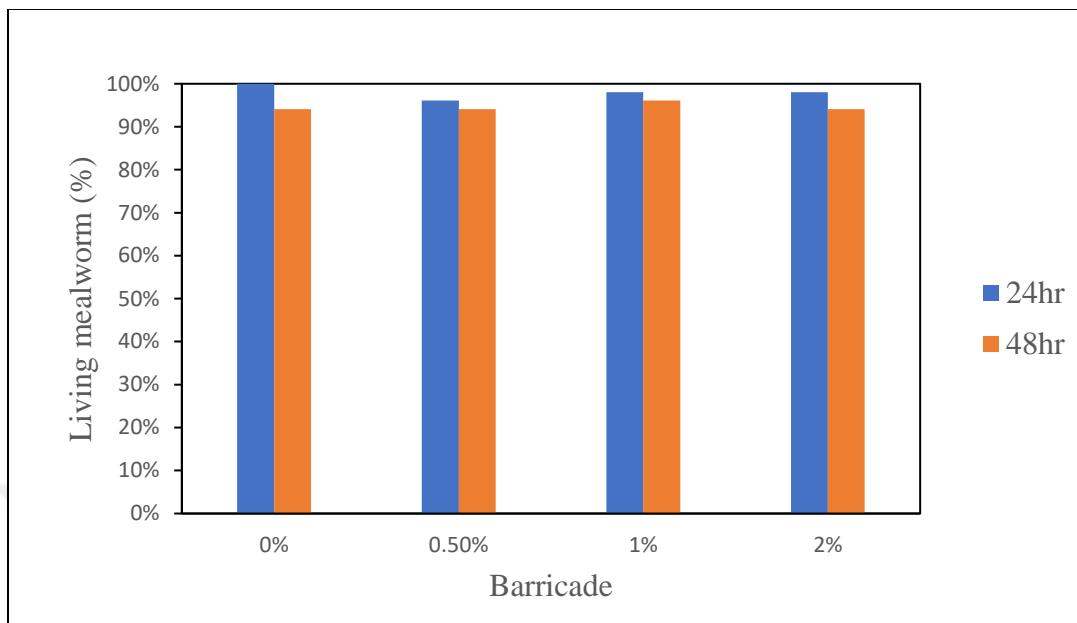


Figure 2.2. Percent living *Tenebrio molitor* larvae (mealworm) after 24 and 48 hr exposure to different concentrations of Barricade® gel. No differences in survival were detected among the treatments. Bars with the same letter are not different according to Student *t* test.

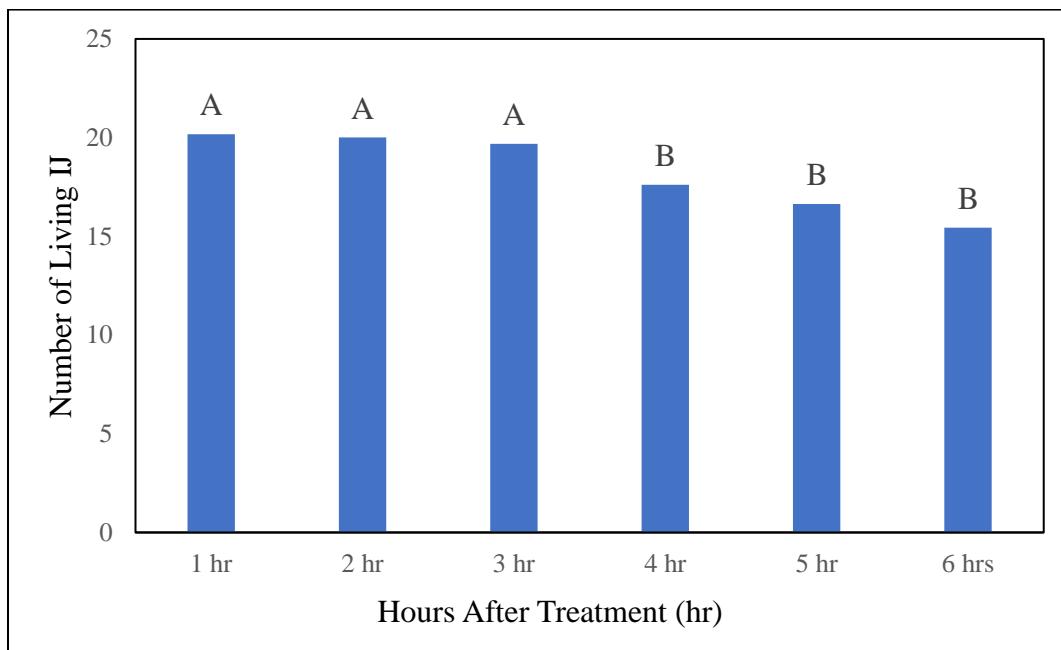


Figure 2.3. Mean number of living *Steinernema feltiae* over 6 hrs of post Barricade® gel treatment. Bars with the same letter are not different according to Students *t* test.

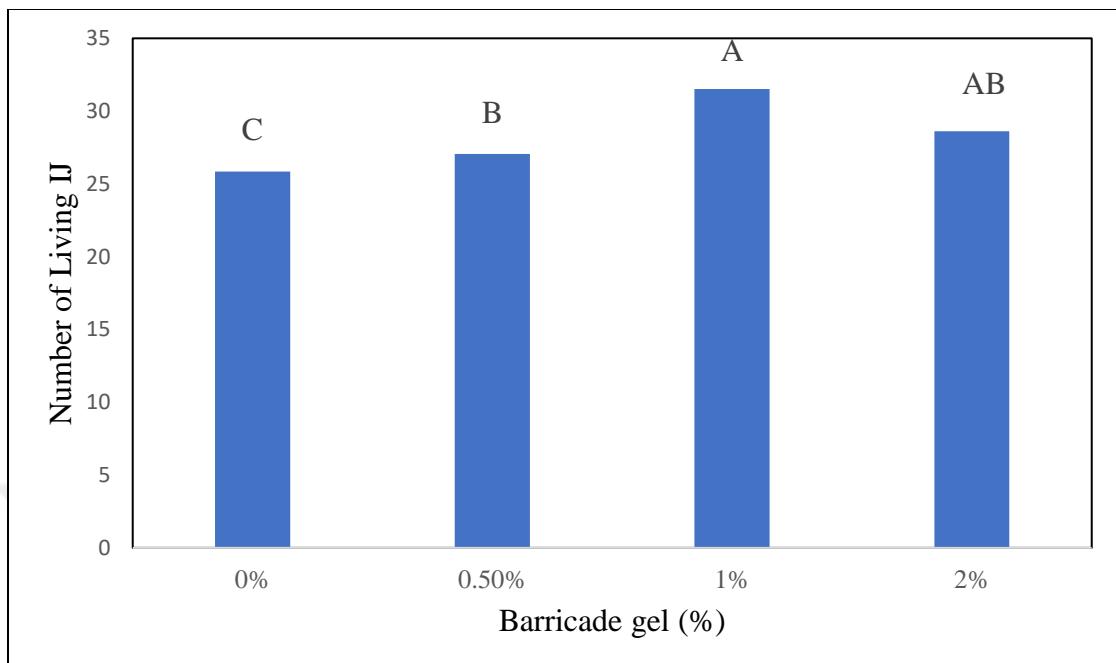


Figure 2.4. Mean number of live *Steinernema feltiae* exposed to different concentrations (0, 0.5, 1 and 2 %) of Barricade® gel. Bars with the same letter are not different according to Students *t* test.

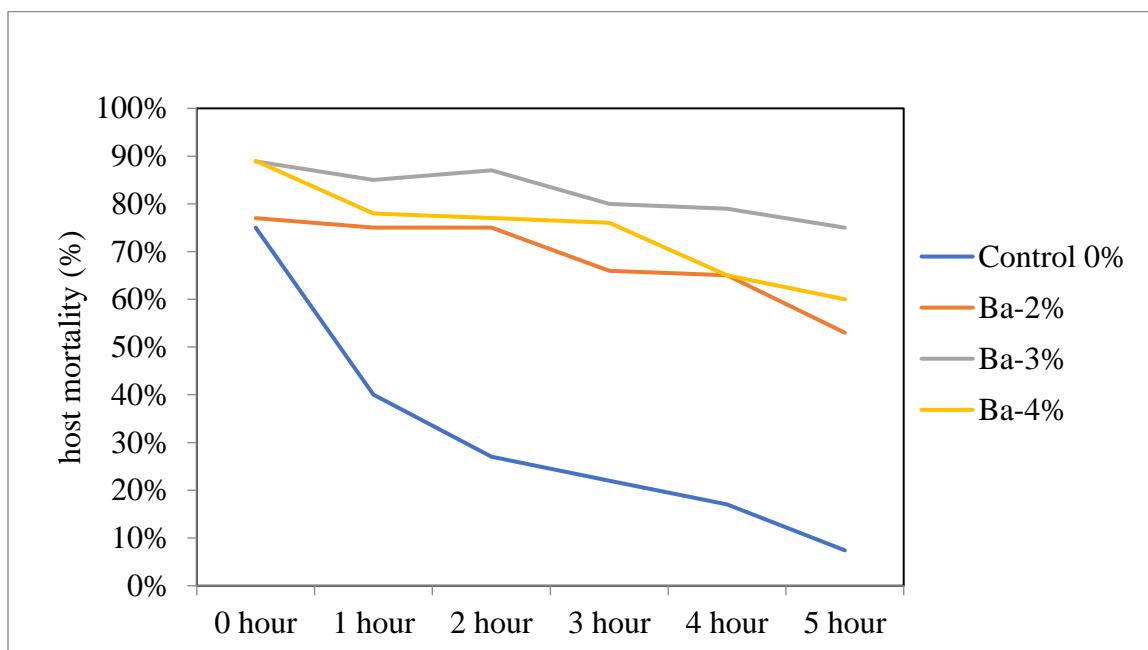


Figure 2.5. Percent mealworm mortality 48 hr after exposure to *Steinernema feltiae* treated with different concentration of Barricade® (Ba) gel for 0 to 5 hrs.

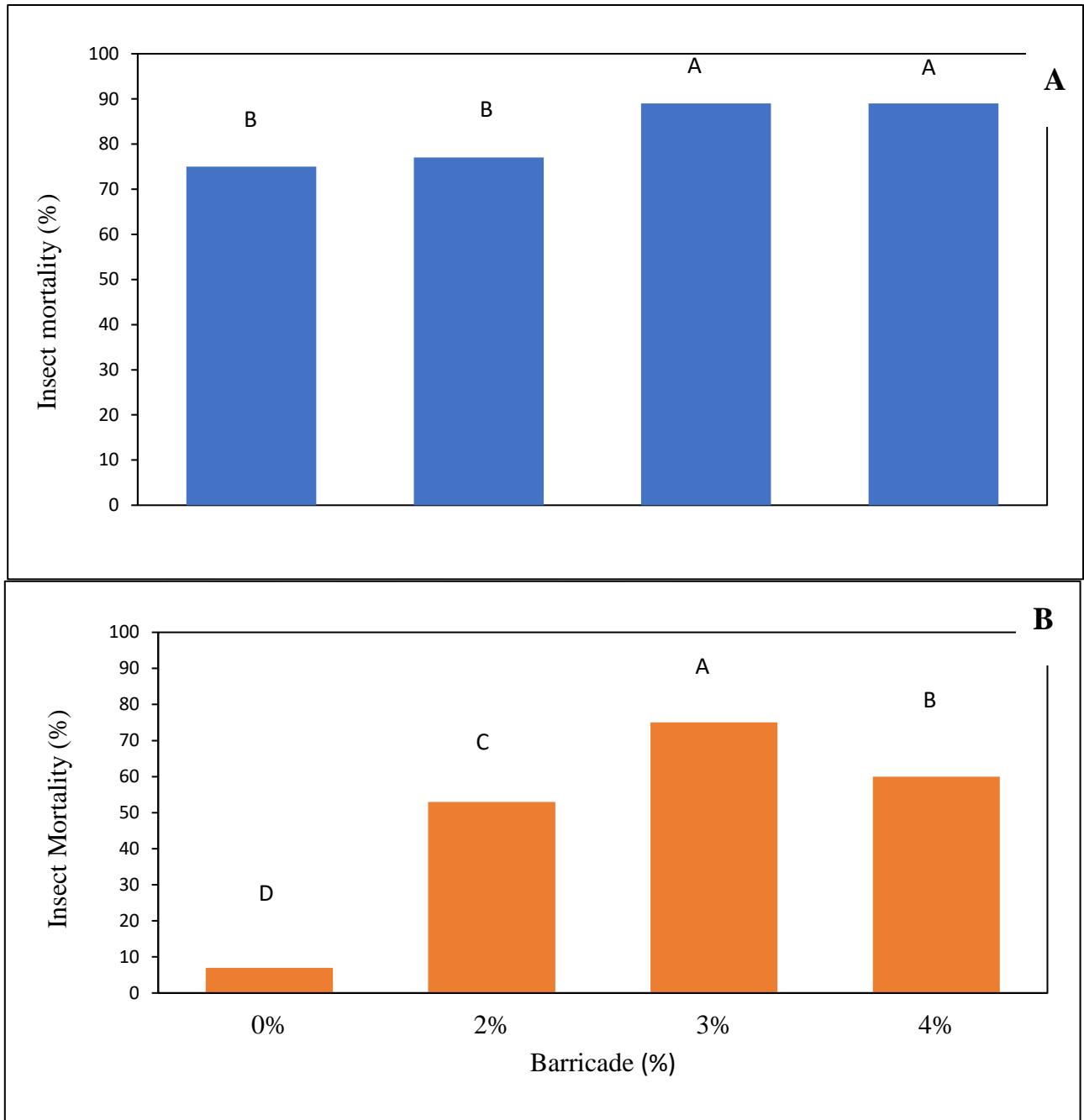


Figure 2.6. Percent of mealworm mortality at 48 hrs after exposure to *Steinernema feltiae* that had been treated with different concentrations (0, 2, 3, and 4%) Barricade® gel and allowed to desiccate for A) 0 and B) 5 hrs. Bars with the same letter are not different according to Student t test.

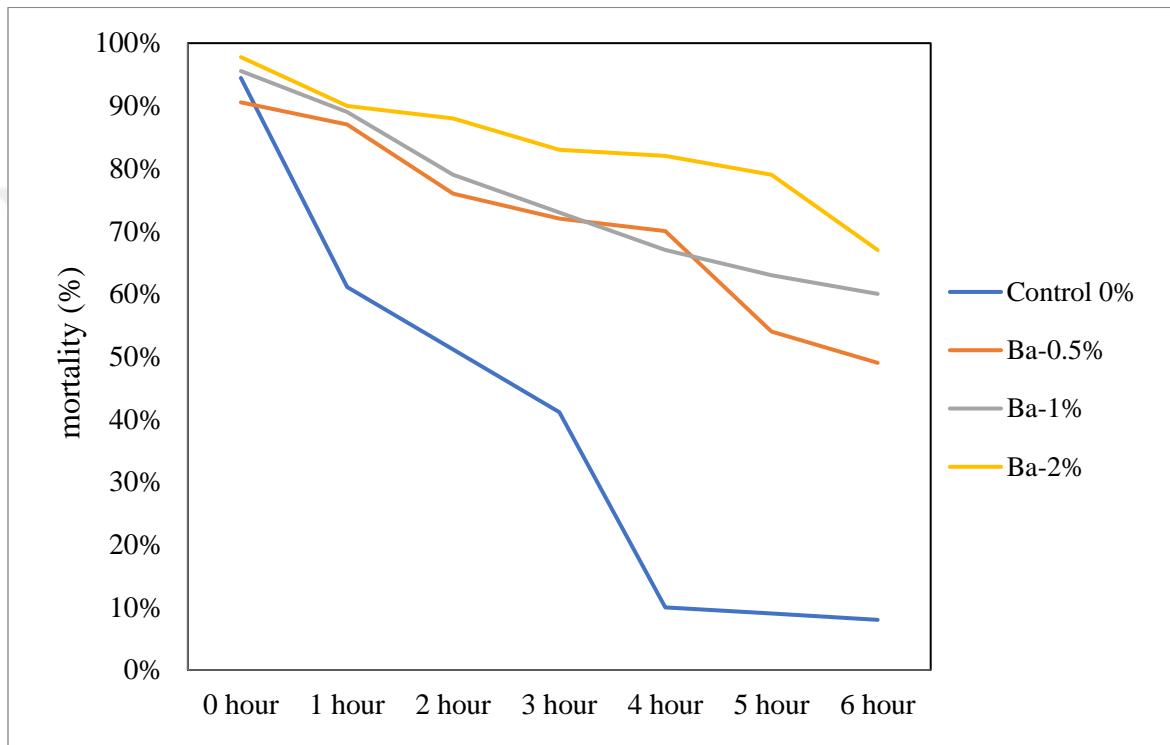
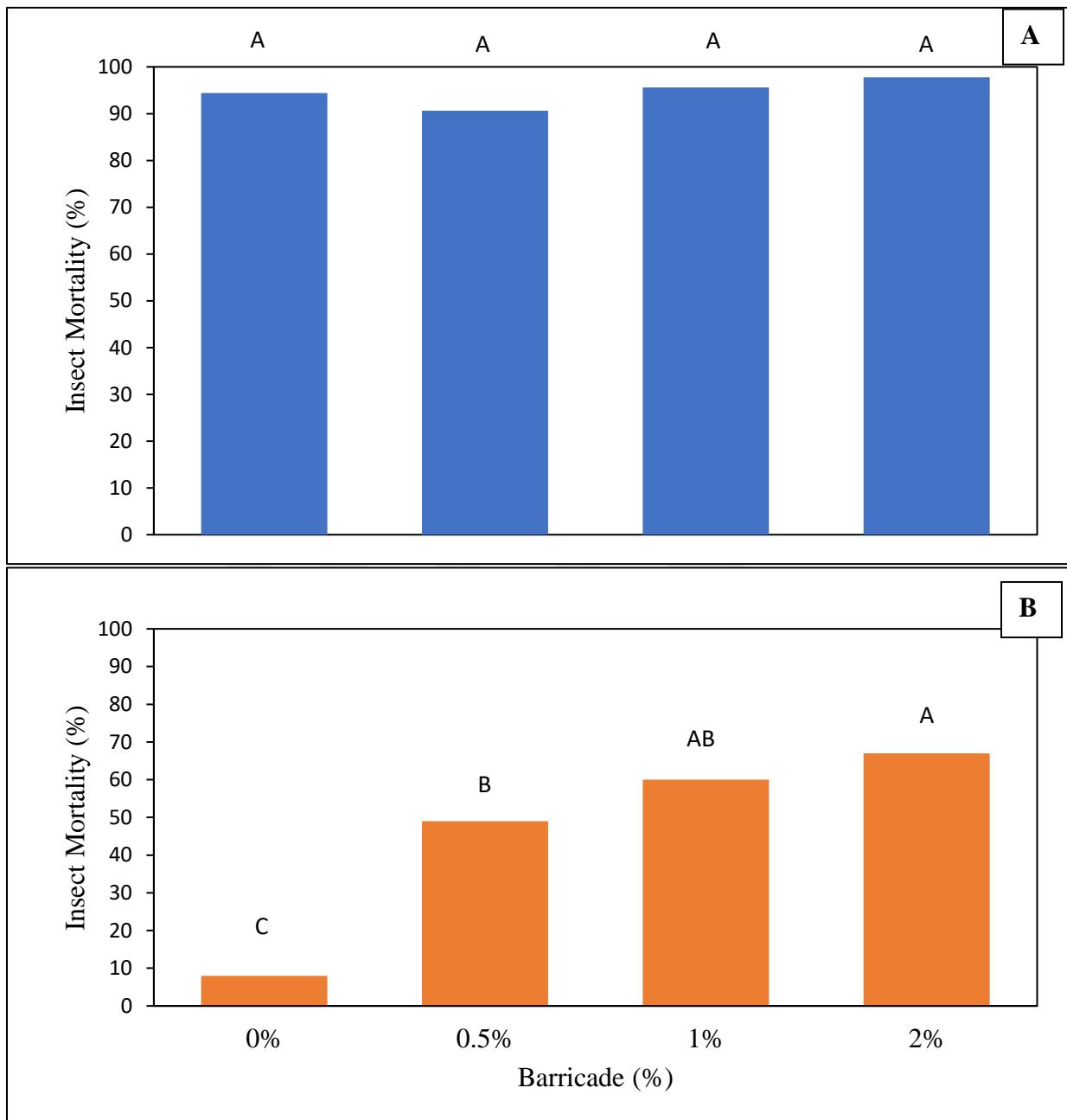


Figure 2.7. Percent mealworm mortality 48 hr after exposure to *Steinernema feltiae* treated with Barricade® gel at 0, 0.5, 1 and 2 % concentration over 6 hours post treatment.



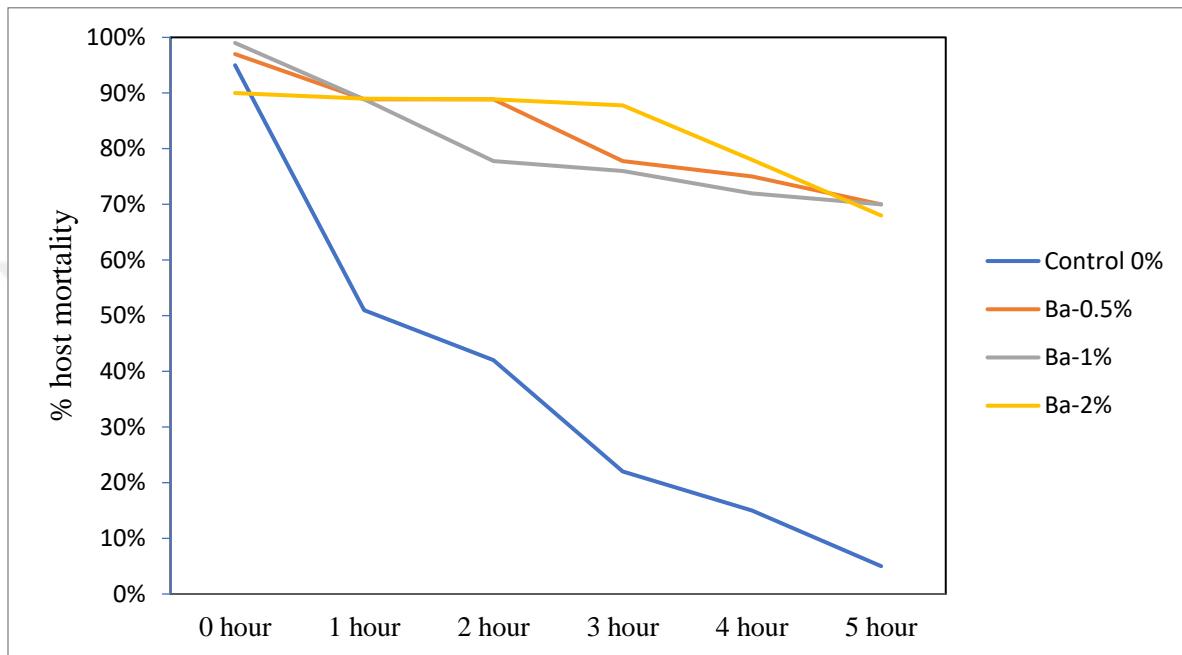
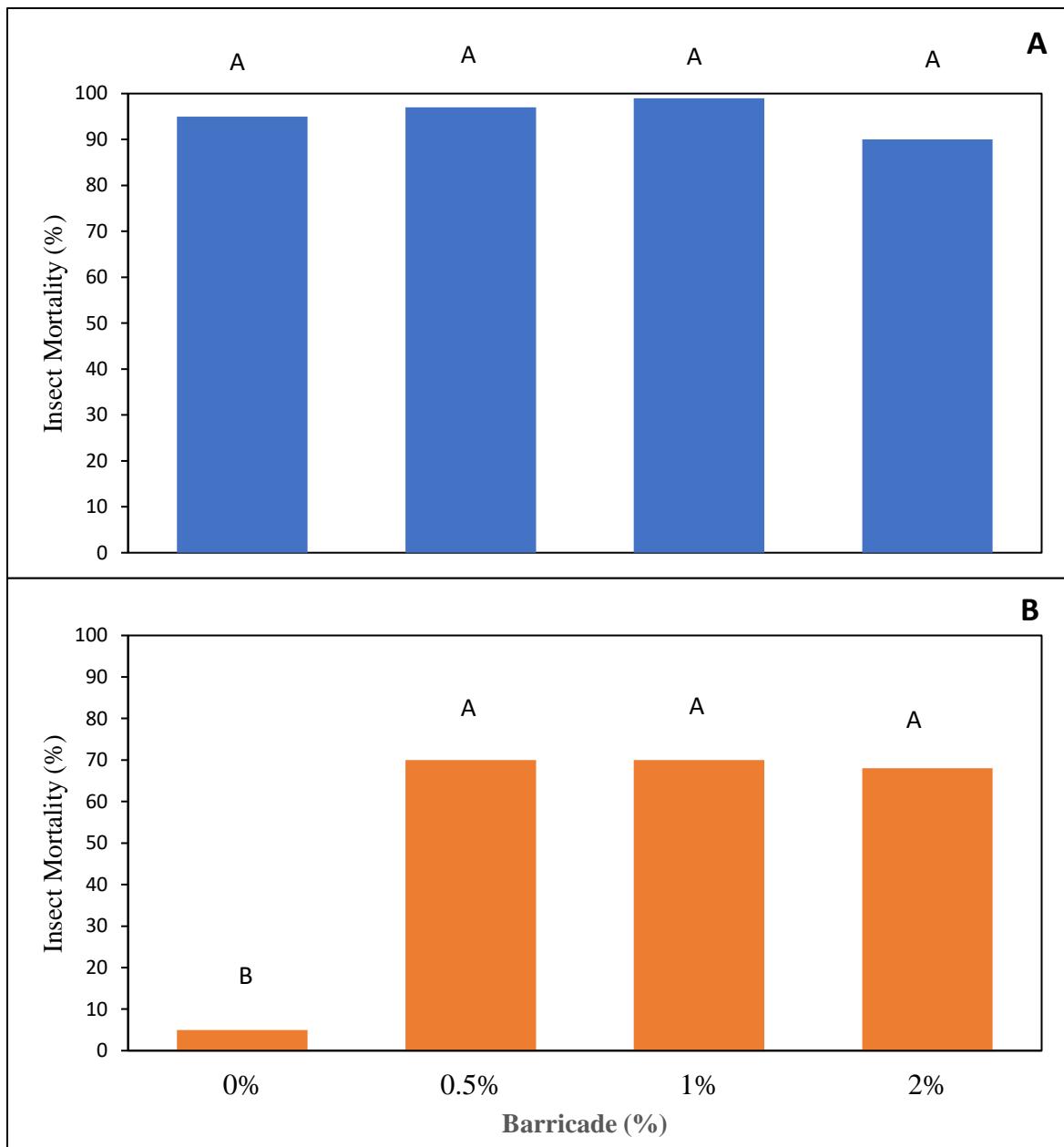


Figure 2.9. Percent mealworm mortality 48 hr after exposure to *Steinernema feltiae* treated with different concentrations of Barricade® (Ba) gel on a choy sum leaf in plate and dried for 0-5 hours.



DISCUSSION

Mealworm larvae are an easy assay insect for assessment of EPN protectants in Hawaii. Mealworm larvae are readily available at pet stores and can be grown in the laboratory easily (Kaya, 1990). Furthermore, Barricade® is not toxic to mealworms.

Barricade® at concentrations as low as 0.5% increased the mortality of the mealworm larvae in the presence of *S. feltiae*. The increased mortality of meal worms is the result of the protection against desiccation that Barricade® provides to the IJ. The protection lasted up to 6 hrs. Concentrations of Barricade® greater than 2% did not provide additional protection to the IJ. At the higher concentrations, Barricade® was difficult to spray and may have adversely affected the IJ and mealworm larvae. Danica *et al.* (2016) showed that lower concentrations of Barricade (0.25-1%) also extended IJ survival rate. Whereas low rates of Barricade® enhance EPN survival, a 2% concentration resulted in greater protection of the IJs and greater subsequent insect mortality.

When applied to leaves, Barricade® also protected EPNs from desiccation. Danica *et al.* (2016) demonstrate that Barricade® allows the IJs to stick to leaf more efficiently than other treatments. Beck *et al.* (2013) showed that EPN deposition and spread on the leaf surface is important to improving the efficacy of foliar application of EPNs. This research demonstrates that Barricade® also protect IJ from desiccation, further enhancing infectivity of IJ.

Application of Barricade® extends IJ survival and enhances infectivity of the insect host. De Waal *et al.* (2013) suggested that at least 4 hrs are critical to ensure that IJs applied above-ground locate and infect insect hosts. Delivery of IJs in a 2% solution of Barricade® can provide the protection for desiccation needed for EPN to infect insect pest that are found above ground. Application IJs in a solution of Barricade® protects against desiccation and therefore enhances IJ

infectivity. Therefore, application of IJ in a Barricade® solution is a viable method to increase the biological control potential of EPN as a pest management tool.

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CHAPTER 3

THE EFFECT OF UV CHEMICAL PROTECTANTS ON *STEINERNEMA FELTiae*

INTRODUCTION

Entomopathogenic nematodes (EPNs) are effective biological control agents of insect pests (Grewal *et al.*, 2005). However, their use as a foliar spray against insect pests is restricted because of the sensitivity of EPN infective juveniles (IJs) to ultraviolet (UV) radiation (Walia and Nandal, 2008; Shapiro-Ilan *et al.*, 2012). UV radiation is a component of the electromagnetic (light) spectrum that reaches the Earth from the sun (Table 3.1). There are three wavelength groups of UV radiation. Wavelengths ranging from 10 nm to 400 nm are classified as UVA, UVB, or UVC. UVA is the longest of the three wavelengths at 320-400 nanometers (nm). UVB ranges from 290 to 320 nm and UVC ranges from 200 to 280 nm. UVC is absorbed by the Earth's ozone layer and does not reach the ground (Epstein and Wang, 2013).

UV radiation is one of the most important abiotic factors that interfere with EPN activity (Gaugler *et al.*, 1992). Exposing EPN to UV radiation can adversely affect the nematode as well as the nematode-associated symbiotic bacterium (Nishimura *et al.*, 1994). Sezhian *et al.*, (1996) reported that mortality of *Spodoptera litura* increased with aboveground application of *Steinernema carpocapsae* in both laboratory and field conditions when the phagostimulant, glycerine and Triton, were added to the suspension of EPNs. Hussaini *et al.* (2003) studied the effect of some optical brighteners (glycerine, liquid paraffin, Triton X-100, Tween 80 and castor oil) as UV protectants and the results showed that optical brighteners reduced the harmful effects of UV radiation on EPNs.

Table 3.1 Ultraviolet (UV) light wavelengths.

Band	Wavelength (Ultraviolet Light)
UV-A	400 - 320 nm
UV-B	320 - 290 nm
UV-C	290 - 100 nm

A range of chemicals are used to block and absorb UV radiation. UV absorbing compounds include P-amino benzoic acid (PABA) and octyl methoxycinnamate (OMC). Congo red, titanium dioxide, and zinc oxide are UV blockers. PABA is widely used in human sunscreens as a UV filter. Hussaini *et al.* (2003) reported that PABA in concentrations of 0.05 and 0.1% offered protection against UV radiation and reduced the harmful effects of UV radiation on the EPN *Steinernema carpocapsae*. OMC is a sunscreen agent that is an organic ester formed from methoxycinnamic acid and 2-ethylhexanol. Congo red is a water-soluble organic benzidine-based anionic diazo dye. Titanium dioxide, also known as titanium (IV) oxide or titania, is a naturally occurring inorganic oxide of titanium, TiO_2 (Dransfield, 2000). Titanium dioxide is insoluble in water. Zinc oxide (ZnO) is an insoluble inorganic compound that occurs in nature as the mineral zincite (Liedekerke, 2006). Application of titanium oxide or zinc oxide on a surface prohibits the UV radiation from moving beyond the chemical blockers, thus protecting against UV radiation.

These chemicals, when used as a UV blocker or absorber, may be able to increase the biological control potential of EPNs against above-ground pests. The objective of this series of experiments was to evaluate the effect of UV chemical protectants on the viability of *S. feltiae*.

MATERIALS AND METHODS

Steinernema feltiae was cultured in commercially obtained mealworm larvae (*Tenebrio molitor*). Ten mealworm larvae were placed in 100-mm-d petri dishes lined with Whatman #1 filter paper. The paper was moistened with 1 ml of nematode suspension containing 1,000 IJ of *S. feltiae* per dish. Additional water was added to ensure the paper was entirely moist. After 48 hrs in the laboratory at 25°C, dead larvae were moved to White traps (White, 1927) and held at 25°C until IJs emerged. Newly emerged IJs were collected daily, pooled, counted, and adjusted to 1,000 IJ/ml. These IJs were stored at 15°C until used in the following studies. No culture used in this experiment were over 30-day old.

In a preliminary experiment, mealworm larvae were exposed to UV protectants PABA, OMC, Congo red, titanium dioxide, and zinc oxide to determine possible toxicity. Five mealworm larvae were placed in 100-mm-d petri dishes lined with Whatman #1 filter paper. The paper was moistened with 1 ml of a 0, 0.5, 1 or 1.5% solution of one UV protectant chemical. After 24 and 48 hrs in the laboratory at 25°C, dead larvae were counted. Each concentration was replicated 4 times per trial. The experiment was repeated three times. Percent mortality was calculated, and data were subjected to analysis of variance (ANOVA) using JMP statistical software (SAS Inc, Cary, NC).

Toxicity: *Steinernema feltiae* IJs were exposed to UV protectant chemicals (PABA, OMC, Congo red, titanium dioxide, and zinc oxide) in the laboratory. Before each experiment, the desired

UV protectant chemicals solutions were made, and 1 ml of each solution was transferred to a 100-mm-d petri plate. A 1 ml aliquot of 1,000 IJ was added to 0, 0.5, 1 and 1.5% UV protectant chemicals solutions. The petri plates were held for 5 hrs in the laboratory at 25°C after which the EPN were evaluated for mortality. Twenty IJs were pipetted into a BPI dish and EPN survival was assessed by a movement response when probed with a dissecting needle (Kaya and Stock, 1997). A single plate served as an observation. Each concentration was tested in 4 plates, with each plate serving as a replication. The experiment was repeated three times. Percent mortality was calculated and analyzed for variance. A Chi-Square test for homogeneity of variance and data analysis and were conducted using JMP statistical software (SAS).

Effects on IJ infection: The effect of UV protectant chemicals (PABA, OMC, Congo red, titanium dioxide, and zinc oxide) on IJ infectivity was determined. Twenty ml solutions of 0, 0.5, 1, and 1.5% UV protectant chemicals containing 1,000 IJ/ml were made and transferred to 100-mm-d petri plates. The IJs in these solutions were exposed to UV light (Eye Hortilux PowerVeg-T5, full spectrums of UVA and UVB) in a biosafety cabinet for 0, 4, 8 and 12 hrs. At each observation hour (0, 4, 8, and 12), 3 ml of solution was collected from the petri plate and sprayed onto a Whitman #1 filter paper placed in a 60-mm-d petri plate. Three mealworm larvae were introduced into each 60-mm-d petri plate. After 48 hrs exposure to the IJs, the number of dead mealworm larvae was determined. Larval death was confirmed by a movement response. Each treatment, represented by a plate containing three mealworm larvae, was replicated four times and the entire experiment was repeated three times. A Chi-Square test for homogeneity of variance among trials and ANOVA were conducted using JMP statistical software (SAS).

RESULTS

Protectant chemicals were not toxic to mealworm larvae (Fig 3.1). All mealworms were alive at 24 hrs after exposure in all concentrations of all UV protectants. No differences were detected among the concentrations of each protection ($P > 0.05$). The number of living larvae was lower at 48 hrs compared to 24 hrs in each chemical ($P \leq 0.001$).

The UV protectant chemicals were also not toxic to IJs (Fig. 3.2A-F). More than 90% of the IJs survived at 5 hrs after exposure to all protectant chemicals except for titanium dioxide where only 88 % survived at 5 hrs after exposure indoor without UV light. No differences were detected among concentrations of each protection chemicals either ($P > 0.05$). However, there was a difference in IJ survival among times of exposure ($P \leq 0.001$).

All UV protective chemicals protected the IJs from UV radiation very well. The IJs exposed to UV radiation infected and killed more mealworm larvae than those exposed to UV radiation but not protected by chemical. PABA afforded the greatest protection to EPN compared to other chemicals. IJs treated with PABA treatments were affected by time and concentration ($P \leq 0.0001$). Mealworm larvae mortality was over 90% 12 hrs after inoculation with IJs treated with all concentrations of PABA. Insect host mortality was affected by time after UV protective chemical treatments ($P \leq 0.0001$). Insect host mortality decreased was parallel with IJ mortality. Mortality was only 5% at 12 hours in the 0% PABA control treatment (Fig.3.3A). OMC offered the second greatest protection for EPNs against UV radiation. Host mortality was over 90% even after 12 hrs exposure to UV radiation at the 1 and 0.5% OMC concentrations. The 0% OMC control treatment cause a rapid loss in EPN effectiveness and 1.5% concentration had between 80 and 90% mealworm mortality but the host mortality changes over time after the chemical treatment ($P \leq$

0.0001). IJs survival rate was reduced in parallel with host mortality. In OMC treatment, mealworms mortality went down to 3% at 12 hours in the control treatment (Fig 3.3E). Titanium dioxide and Congo red had host mortality between 80 and 90% with all concentration except for the control treatment ($P \leq 0.0001$) (Figs 3.3B and 3.3D). All Congo red treatments were significantly affected by time ($P \leq 0.005$). But no statistical difference was detected by concentration on Congo red treatments ($P > 0.05$). Zinc oxide had insect mortality between 65 and 90% at all concentrations except the 0% control treatment ($P > 0.05$) (Fig. 3.3C).

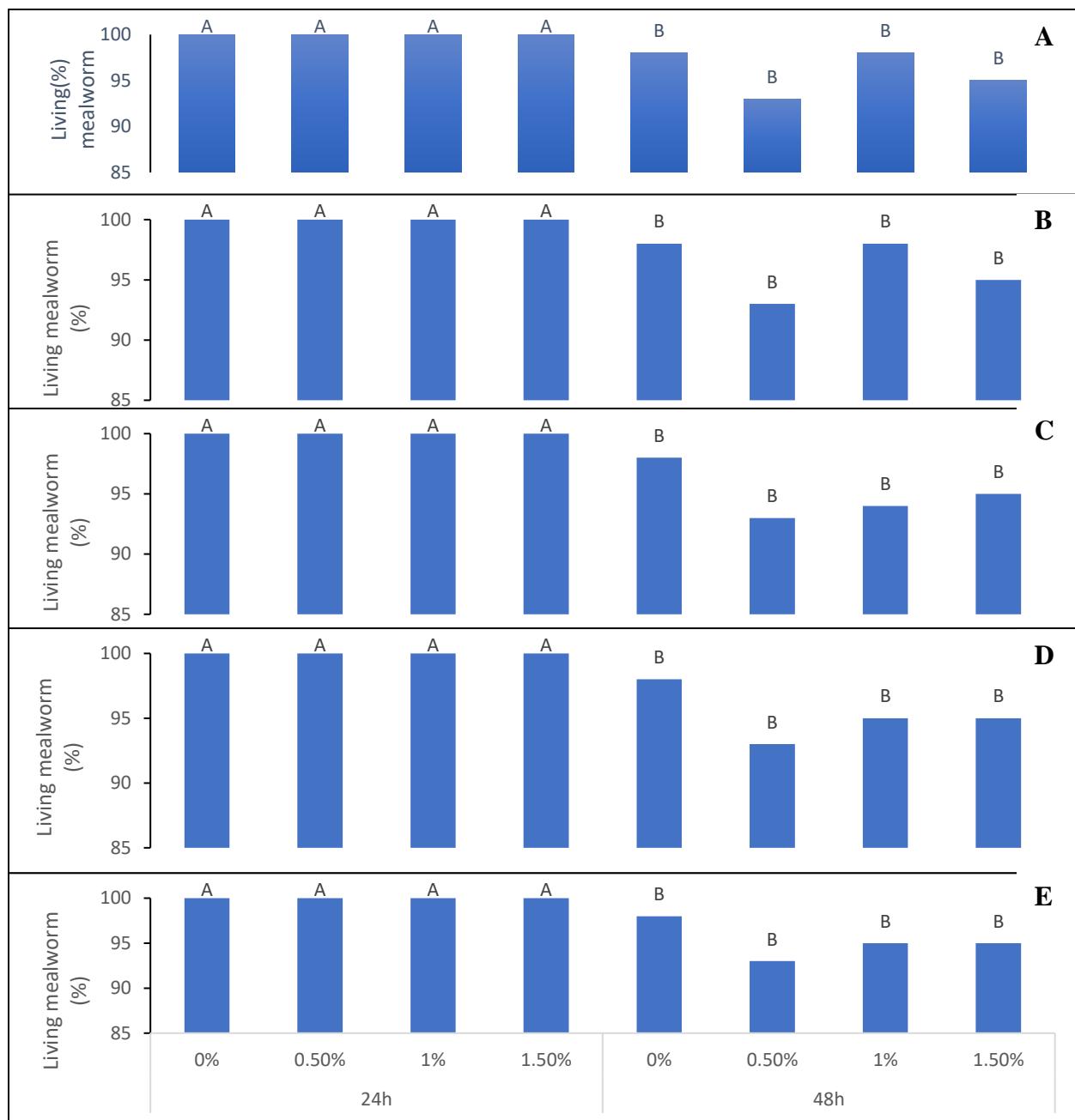


Figure 3.1. Percent of *Tenebrio molitor* larvae (mealworm) survive 24 and 48 hours after exposure to different concentrations of Para-Aminobenzoic Acid (PABA) (A), titanium dioxide (B), zinc oxide (C), Congo red (D), and octyl methoxycinnamate (E). No differences in mealworm survival were detected among the concentrations but there was difference between times according to Student *t* test.

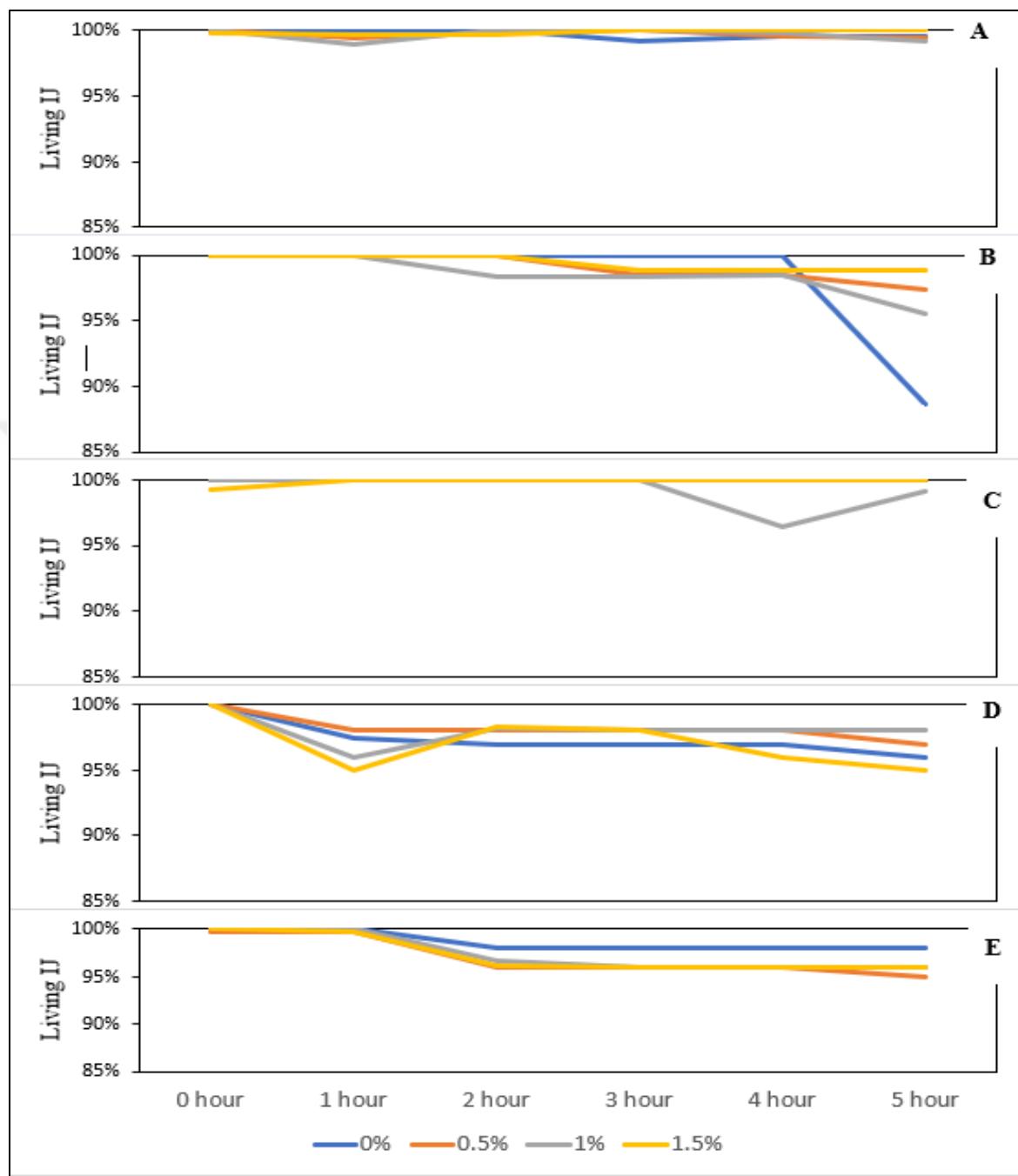


Figure 3.2. Percent living IJs of *Steinernema feltiae* over 5 hours exposure to Para-Aminobenzoic Acid (PABA) (A), titanium dioxide (B), zinc oxide (C), Congo red (D), and octyl methoxycinnamate (E). at different concentrations of 0, 0.5, 1 and 1.5 %. No differences in survival were detected among the concentration but there was difference among time according to Student t test.

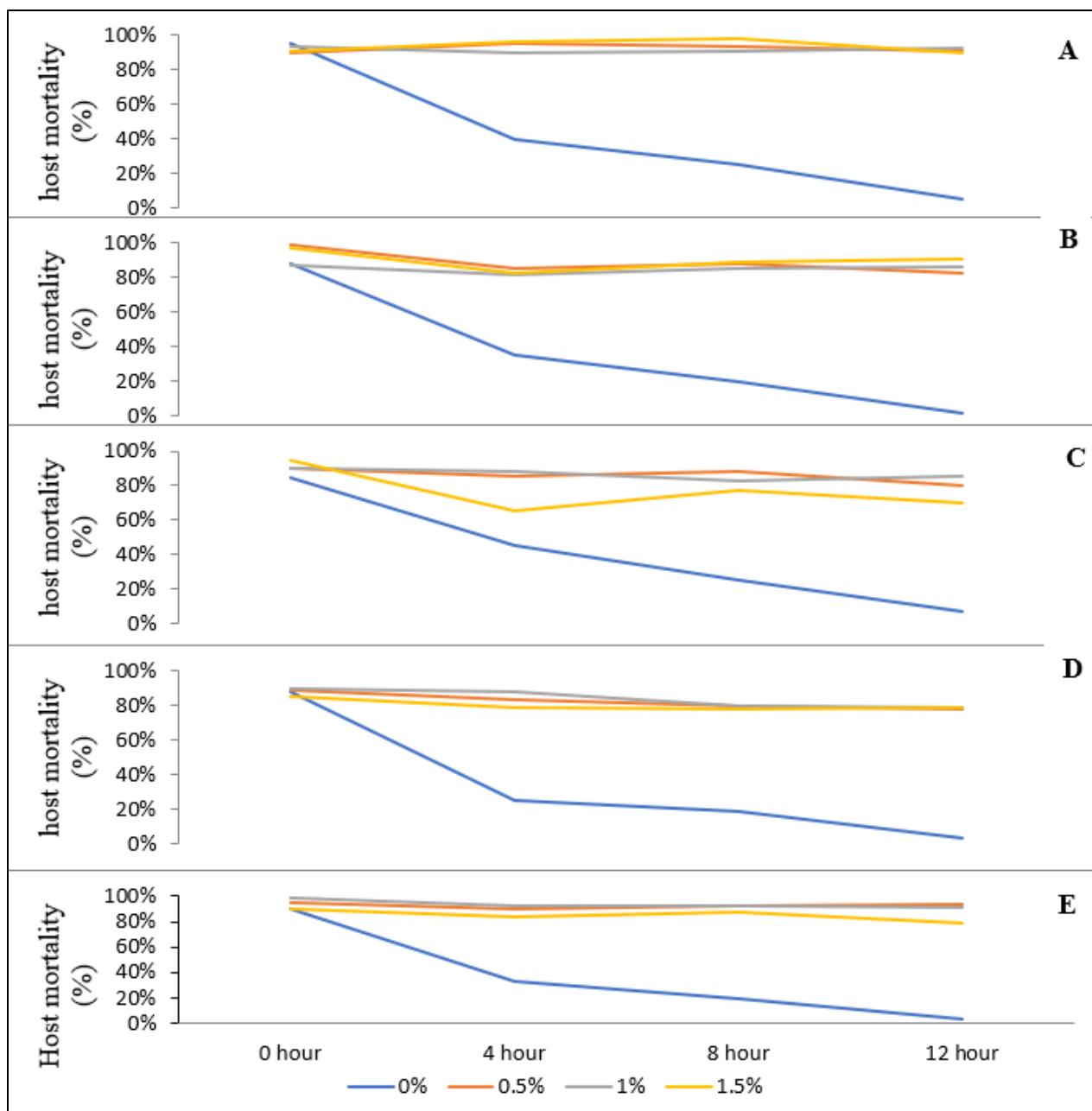


Figure 3.3. Percent mealworm mortality at 0, 4, 8 and 12 hrs after exposure to UV light *Steinernema feltiae* treated with PABA (A), titanium dioxide (B), zinc oxide (C), Congo red(D), and octyl methoxycinnamate (E) at different concentrations of 0, 0.5, 1 and 1.5 %. No differences in host mortality were detected among the concentration but there was difference among time according to Student t test.

DISCUSSION

UV protection can be afforded to EPN in multiple ways. Some chemicals block UV radiation whereas others absorb UV radiation. The most desirable protection for foliar application of EPN would be a chemical that absorbs UV radiation.

Walia *et al.* (2008) demonstrated that UV protection was only 30-40% in zinc oxide and 100% in titanium dioxide but only at higher concentrations. However, zinc oxide and titanium dioxide provided UV protection for IJs at a lower concentration in this experiment. Shapiro (1989) demonstrated that Congo red provided protection from UV radiation at a 1% concentration. These substances however are insoluble in water and coat the IJ. If applied to shoots, the foliage would also be covered, and this may hamper photosynthesis. Consequently, zinc oxide, titanium dioxide, and Congo red were not desirable for foliar IJ applications.

PABA and OMC can be prepared into clear solution that are also effective in absorbing UV radiation. PABA is soluble in water and very effective in reducing the harmful effect of UV radiation on *S. feltiae* and other EPNs (Hussaini *et al.*, 2003, Gaugler and Boush, 1979). A 1% PABA solution was most effective concentration compared to other concentration in this experiment. OMC can be suspended in a water solution. Danica *et al.* (2016) showed that OMC protected EPNs from sun exposure over 120 min, with 80% of EPNs remained viable. Current OMC experiment was consistent with this result. OMC protected IJ of *S. feltiae* against UV radiation and had the second highest insect mortality at a 1% concentration. Walia *et al.* (2008) also demonstrated that PABA and OMC have greater utility for foliar applications because they may not interfere with photosynthesis while protecting the IJs.

The efficacy of IJs is improved with the incorporation of UV protectants. IJ infectivity can be maintained at high levels for up to 12 hrs. It is feasible to apply IJ during normal working hours rather than at dusk if the spray mix includes an UV protectant.

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CHAPTER 4
ENHANCED INSECT CONTROL WITH EPNS PROTECTED
FROM
UV RADIATION AND DESICCATION

INTRODUCTION

Entomopathogenic nematodes (EPNs) are biological control agents that can be deployed against a variety of economically important insect pests (Grewal *et al.*, 2005). However ultraviolet radiation (UV) and desiccation can limit the efficacy of EPNs when applied to foliage (Shapiro-Ilan *et al.*, 2002). The survival of *Steinernema kushidai* was adversely affected by sunlight (Fujii and Yokoyama, 1998) such that the number of *Actinodoria cuprea* larvae infected by *S. kushidai* and the number of cadavers producing nematode progeny significantly decreased after exposure to desiccation and UV light.

Foliar applications of EPNs have been a challenge to be used as biological control agents. The poor efficacy of foliar applications of EPNs is associated with UV radiation and desiccation (Arturs *et al.*, 2004). Fujii and Yokoyama (1998) demonstrated that UV exposure harmed the nematode's symbiotic bacteria as well as the nematodes themselves. Nickle and Shapiro (1994) demonstrated that formulation of the IJ solution could preserve infectivity of *S. carpocapsae* up to 95% after 4 hours of exposure to direct sunlight. As a result, various formulations have been tested to enhance the efficacy of EPN applications (Van Niekerk and Malan, 2015).

Improving environmental conditions for the EPNs will increase their efficacy as biological control agents. Formulation and application technology is one approach to increasing the efficacy of aboveground EPN applications (Glazer *et al.*, 1992; Baur *et al.*, 1997; Head *et al.*, 2004).

Protection from ultraviolet (UV) light is equally important for the survival of EPN IJs. IJs are typically soil inhabiting organisms, and thus have no tolerance to UV radiation. However, Hussaini *et al.* (2003) reported that PABA in concentrations of 0.05 and 0.1% offered protection against UV radiation and blocked the harmful effects of UV radiation on *S. carpocapsae*.

The objective of this experiment was to determine the effect of protecting IJ of *Steinernema feltiae* from UV radiation and desiccation on their ability to infect and kill insects.

MATERIALS AND METHODS

Steinernema feltiae was cultured in commercially obtained mealworm larvae (*Tenebrio molitor*). Ten mealworm larvae were placed in 100-mm-d petri dishes lined with Whatman #1 filter paper. The paper was moistened with 1 ml of a solution containing 1000 IJ of *S. feltiae* per dish. Additional water was added to ensure the paper was entirely moist. After 48 hrs in the laboratory at 25°C, dead larvae were moved to White traps (White, 1927) and held at 25°C until IJs emerged. Newly emerged IJs were collected daily, pooled, counted, and adjusted to 1,000 IJ/ml. These IJs were stored at 15°C until used in the following studies. No EPN cultures were over 30 days old.

A series of experiments was conducted to evaluate the ability of solutions containing UV radiation and desiccation protectants to protect and enhance IJ infection of insects. Solutions (20 ml volumes) of UV protectants P-amino benzoic acid (PABA) and octyl methoxycinnamate (OMC) were individually combined with Barricade® fire gel and used to deliver IJs. In the first experiment, solutions of 1% UV protectant chemicals containing 1000 IJ/ml were made and combined with 3% Barricade® gel. These solutions (PABA+Barricade, OMC+Barricade, Barricade only, and a water control) were poured into 60-mm-d petri dish, left open and exposed

to UV light (Eye Hortilux PowerVeg-T5, full spectrums of UVA and UVB) in a biosafety cabinet for 0, 6 and 12 hrs. After the desired exposure time (0, 6, or 12 hrs), the solution was transferred into a small plastic spray bottle and sprayed onto a sterile 60-mm-d petri plate lined with Whitman #1 filter paper. Three mealworm larvae were introduced into each dish. In a second experiment, similar IJ solutions were made and exposed to UV radiation in open containers in a biosafety cabinet with the IJ spayed onto a single leaf of *Brassica chinensis* var. *parachinensis* (choy sum) that was placed in a 60-mm-d petri dish.

In both experiments, after 48 hrs exposure to the IJ, the number of dead mealworm larvae in a petri dish was determined. Larval death was confirmed by a movement response. Each treatment, represented by a plate containing three mealworm larvae, was replicated four times and the entire experiment was repeated three times. A Chi-Square test for homogeneity of variance among trials were conducted. Analysis of variance among treatments were conducted using JMP statistical software (SAS). The data were found to have similar variances and were combined for further analysis. A repeated measures analysis was conducted on the data.

RESULTS

The combination treatments of OMC+Barricade and PABA+Barricade afforded the greatest protection to IJs from UV radiation and desiccation in the filter paper experiment. All insects died (100% mortalit) when the IJ had not been exposed to UV radiation or any desiccation ($P \leq 0.01$) (Fig. 4.1). After exposure to UV radiation, host mortality decreased in all treatments (Fig. 4.1). With a 6 hr exposure to UV radiation and 48 hr desiccation, unprotected IJ infection of mealworm decrease substantially compared to the other treatments (Fig. 4.1). Infectivity of IJs in solutions containing a UV and desiccant protectant were among the highest ($P \leq 0.0001$).

throughout the 12 hours of observation time (Fig. 4.1) compare to the other treatments. Survival rate and infectivity of IJs in the Barricade® only treatment was intermediate between no protection (water control) and the UV and desiccant protection treatments at both 6- and 12-hour observation times. These Barricade® only treated IJ incited 70% host mortality after 12 hours (Fig. 4.1). However, the host mortality recorded with the IJ in the water control treatment decreased sharply with exposure to UV radiation and achieved only 6% mealworm mortality at the 12 hr of exposure.

In the second experiment conducted on choi sum leaves, a similar behavior was observed (Fig. 4.2). Before exposure to UV radiation, all IJ treatments achieved host mortality between 90 and 100%. However, after 6 hrs UV exposure, unprotected IJs resulted in less than 50% mortality of the mealworm larvae. The Barricade® only treatment resulted in greater insect mortality than the water control ($P < 0.01$), whereas Barricade+OMC and Barricade+PABA combinations provided the greatest UV radiation protection to IJs (Fig. 4.2). After 12 hrs of exposure, the protection from OMC and PABA allowed IJs to kill 80% of the mealworm larvae (Fig. 4.2). Barricade® itself provided UV protection to the IJ resulting in 76% mealworm larvae mortality after 6 hrs and 63% mortality after 12 hours (Fig. 4.2). The choy sum leaves dried out quickly and curled up in the laboratory conditions, especially in the water only treatment. The leaves receiving the Barricade® spray curled up less and experienced less drying out. This curling may have negatively impacted IJ infection rate on the mealworm larvae once exposed to the UV light.

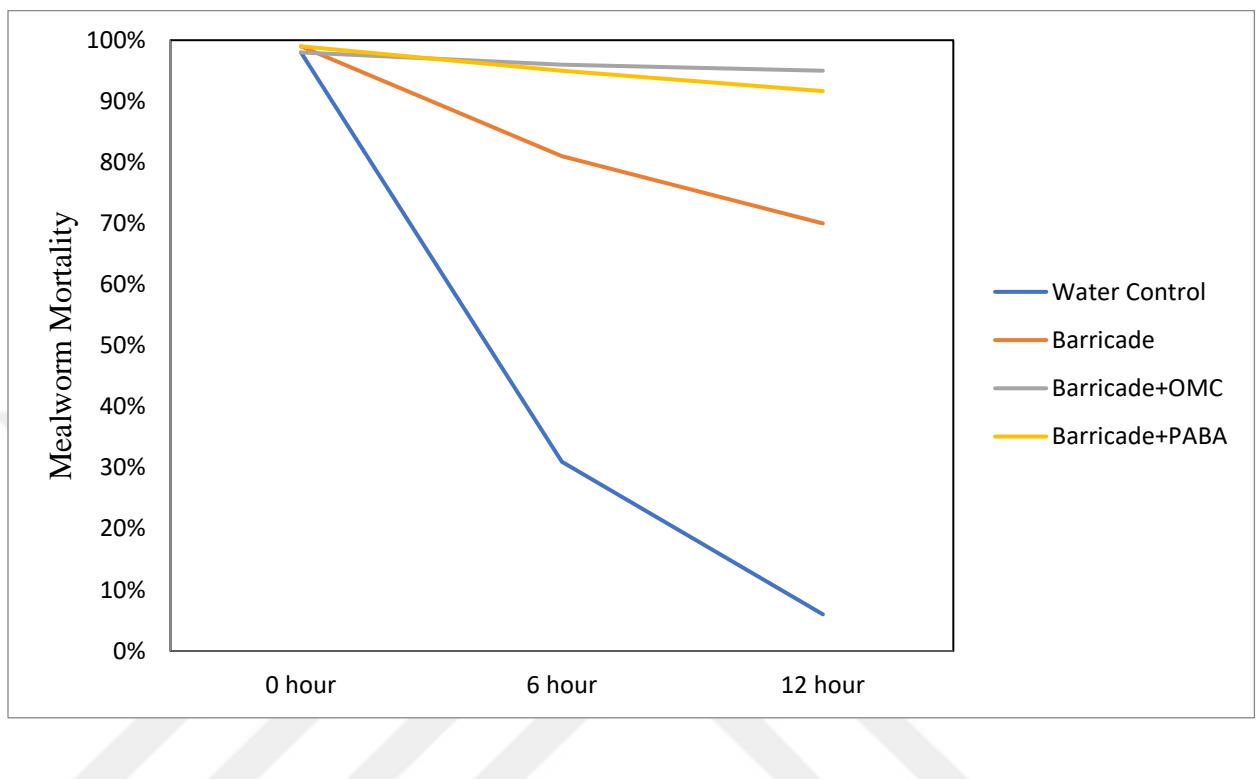


Figure 4.1. Percent mealworm mortality at 48 hours after introduction to *Steinernema feltiae* when exposed to UV light for 0, 6 or 12 hrs. The infective juveniles (IJs) were suspended in a solution of the desiccant protectant Barricade, Barricade[®] with the UV radiation protectant octyl methoxycinnamate (OMC), Barricade[®] with the UV radiation protectant P-amino benzoic acid (PABA), or water. IJs were applied to filter paper after UV exposure before introduction of mealworm larvae.

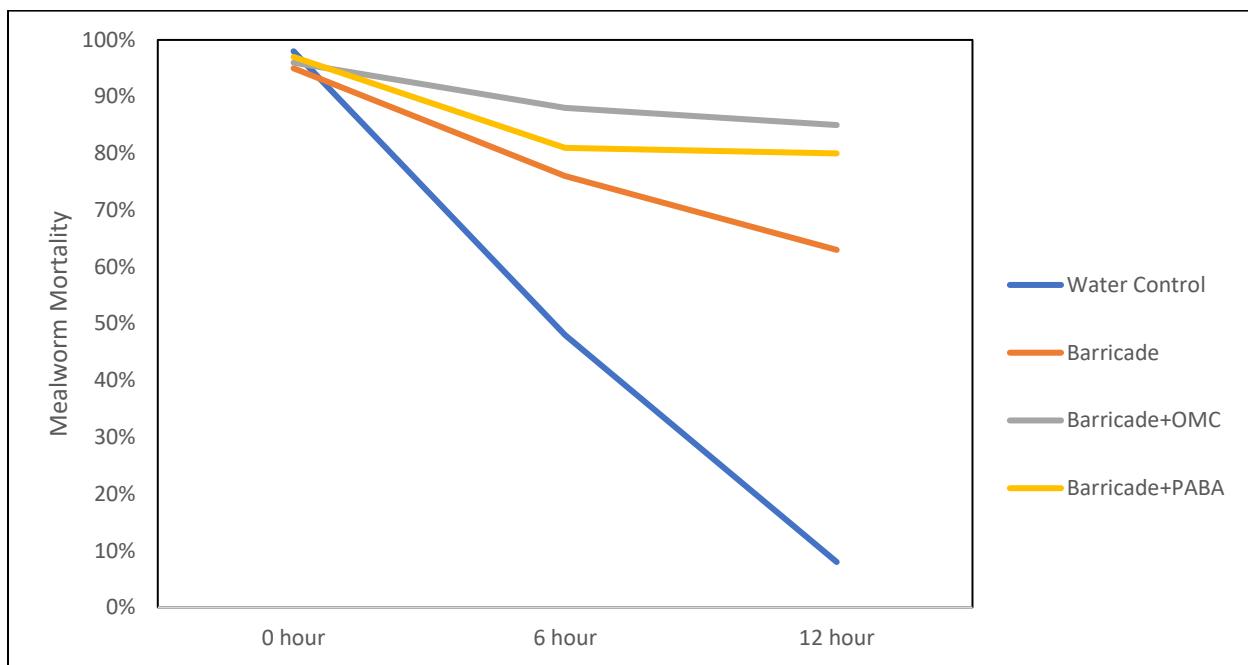


Figure 4.2. Percent mealworm mortality on choy sum leaves from infection by *Steinernema feltiae* applied in a solution of the desiccant protectant Barricade®; Barricade® and the UV radiation protectant octyl methoxycinnamate (OMC); Barricade® and the UV radiation protectant P-amino benzoic acid (PABA); or water after 0, 6 and 12 hrs after exposure to UV radiation.

DISCUSSION

EPNs can be protected from UV radiation and desiccation and this protection can improve insect control. The combination of a desiccant protectant, such as Barricade®, with a UV radiation protectant such as PABA or OMC provides very high levels of protection to the IJ of EPN resulting in substantial insect larvae mortality. Between 80-95% mortality was achievable with the combination treatments, acceptable percentages for control of foliar insect pests. Without UV radiation protection, IJs failed to induce host mortality after exposure to UV radiation.

In order to improve the efficacy of EPNs foliar applications, we can take advantage of the combined desiccant and UV protectant. Adding these protectants at foliar application of EPNs will minimize or eliminate the need to recommend evening application of EPN for greatest efficacy. The formulation of EPN with UV and desiccant protectants could allow for more widespread use of nematodes as biological control agents.

These combinations (UV protectants plus Barricade®) should be tested in the field with a targeted economically important insect pests so as to observe environmental effect of EPNs protected by UV protectants plus Barricade® on a crop. Future research should examine if UV protectants plus Barricade® would affect photosynthesis of crop and how it might affect crop yield. While current research show no impact of the combined UV protectants and Barricade® gel over 6 hours, future research should examine how to integrate the formulation of commercial EPN inoculum with these protectants. It might be possible to formulate a supplemental protectant solution with the best combination of Barricade® gel with 1% concentration of PABA. The goal is to only combine commercial EPNs with the protectant in water prior to field application.

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